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INDIVIDUAL AND COMBINED TOXICITY OF NITRILES AND ALDEHYDES TO *RAPHIDOCELIS SUBCAPITATA*

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Abstract—A closed-system algal toxicity test with no headspace was applied to evaluate the toxicity of 11 aldehydes and two nitriles to *Raphidocelis subcapitata*. Algal growth rate and dissolved oxygen (DO) production were used as the test endpoints. Compared to literature data, our test results based on the endpoint of DO production is 2.5 to 257 times more sensitive than the conventional algal batch tests. In addition, our analyses show that different relative toxicity relationships may be observed when different test methods or different endpoints are applied. The test alga was found to be quite sensitive to aldehydes, and a quantitative structure-activity relationship (QSAR) relationship was established based on the *n*-octanol/water partition coefficient (log K_{ow}). This study also evaluated the combined effects of aldehydes and nitriles. Four synergistic joint actions related to malononitrile were identified. On the other hand, most of the combined effects between aldehydes and acetonitrile were antagonistic. In general, we find that greater-than-additive effects mainly are associated with toxicants displaying flat dose-response curves, and less-than-additive effects may be related to certain steep-slope chemicals. Model analyses show that the above mixture toxicity behavior may be due to response addition or response multiplication joint action modes.

Keywords—Algae *Raphidocelis subcapitata* Median lethal concentration Aldehyde Mixture toxicity

INTRODUCTION

Microalgae have been used extensively for assessing the relative toxicity of chemicals and/or waste discharges. The batch technique traditionally is adopted by most standard algal test protocols for actual practice purposes [1–4]. Several studies indicate that algal toxicity tests reveal excellent sensitivity to heavy metals but were relatively insensitive to organic toxicants [5–7]. For algal toxicity tests, the main reason causing the low sensitivity to organic toxicants can be related to the open test environment and vigorous mixing usually employed by these protocols. This experimental design causes the loss of volatile organic toxicants and, consequently, underestimation of the toxicity of volatile organic chemicals. Several studies solved the above problem by adopting a closed system and providing large headspace as additional carbon source for algal growth [8–10]. However, the complicated experimental design is still the main drawback of the above approach. Furthermore, previous research also indicates that headspace may cause the inaccuracy of concentration estimation and lower test sensitivity [11]. To overcome this, we recently developed a closed-system algal toxicity test technique with no headspace ([12], http://www.enviroaust.net/). The experimental design is quite simple and the test revealed satisfactory sensitivities to both metallic and organic toxicants.

Research efforts on the toxicology of chemical mixtures have existed for several decades. The major aims of these studies were to explore ways to predict and to identify hazardous combinations of chemicals relevant to humans and the environment. The fundamental development of multiple toxicity theory was made by Bliss [13] who defined two basic reaction modes for joint toxicity: Similar joint action and independent joint action. Hewlett and Plackett [14] later presented a more comprehensive approach that unified the basic modes in a general model based on a bivariate normal distribution of the action tolerances. Their model has a noninteractive nature, meaning that the response of one toxicant does not affect the combination of another with receptors or the intrinsic activity of the other [15]. Christensen and Chen [16] further expanded the model to introduce *n* toxicants and an arbitrary tolerance distribution.

The development of the narcosis quantitative structure-activity relationships (QSARs) has led to a general classification of organic chemicals into nonreactive and reactive types. Reactive toxicants further have been divided into four different categories according to their mechanisms of toxicity [17]. The QSARs have been applied to discriminate between chemicals having similar and dissimilar mechanisms of toxicity. Chemicals belonging to the same QSAR group are considered to have the same mechanism, and their combined toxic effects have been found to be additive [18–23]. Furthermore, the joint actions between narcotic chemicals (nonreactive toxicants) are found to be either additive or less than additive [24]. On the other hand, data based on binary mixtures of organic toxicants indicates that a considerable proportion of mixtures of reactive toxicants displayed greater-than-additive effects. Most synergistic joint actions observed were related to reactive toxicants having different mechanisms of toxicity and flat concentration-response curves. In particular, aldehydes and nitriles have a high tendency to react synergistically [25,26]. Chen and Lu [27] also demonstrated that, for a specific chemical mixture, different joint action modes were revealed by *Escherichia coli* and luminescent bacteria. Although the two kinds of test organisms responded quite differently to mixtures of organic chemicals having different mechanisms, their multiple toxicity behaviors can be described adequately by the response addition mode indicating a negative correlation of tolerances.

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Table 1. Definitions of basic modes of action

Parameter					
ρ[a]	λ[b]	Type of action	Abbreviation	Response[c]	Effect
1	1	Concentration addition	CA[d]	—	Additive
0	0	Response multiplication	RM	$1 - (1 - P_1)(1 - P_2)$	—
1	0	No addition	NA	Max (P_1, P_2)	Antagonistic
−1	0	Response addition	RA	Min (1, $P_1 + P_2$)	—

[a] ρ = Correlation coefficient.
[b] λ = similarity coefficient.
[c] P_1, P_2 = responses for toxicant 1 and 2 when applied individually.
[d] CA = Concentration addition, response multiplication (RM), no addition (NA), and response addition (RA).

The majority of these reports were based on results from fish, microinvertebrate, and bacterial tests. Due to the aforementioned shortcomings of batch-type algal toxicity tests, the individual and combined toxicity of organic chemicals on microalgae rarely have been investigated. The objectives of this study were to evaluate the individual and combined effects of aldehydes and nitriles using an airtight algal toxicity test with no headspace and to provide model analyses on the observed combined effects based on a noninteractive multiple toxicity model.

The Weibull model was applied herein to describe the tolerance distributions of the test alga to individual toxicants [28], along with a bivariate density function to describe the distribution of toxicant tolerances to two toxicants [16,29]. The bivariate model has two parameters, λ and ρ. The similarity coefficient λ measures the degree of similarity between the actions of two toxicants: λ = 1 indicates that two toxicants act on the same biological system (similar joint action), and λ = 0 indicates that two toxicants act on different biological systems (independent joint action). The correlation coefficient ρ of the bivariate density function measures the degree of linear association between toxicant tolerances. A value of ρ = 1 (or −1) indicates that the tolerances are fully correlated, and ρ = 0 indicates zero correlation of these variables.

This noninteractive model can generate unique cases of joint action modes, e.g., concentration addition (CA), response multiplication (RM), no addition (NA), and response addition (RA) (Table 1). A mixture acting via concentration addition (CA) often is more toxic than acting via RM or NA [30]. The CA model has been recommended for the prediction of the combined effects of mixtures of toxicants [31]. However, previous work demonstrated that, for toxicants having flat dose-response curves, RA and RM modes may result in more severe (synergistic) combined effects than that from the CA mode [27,29].

The additive index (M), or the sum of toxic units that determines the type of joint action for a specific binary mixture of toxicants, is defined by the following equation:

$$M = \frac{z_1}{\mathrm{EC50}_1} + \frac{z_2}{\mathrm{EC50}_2} \tag{1}$$

where z_1 denotes the toxicant concentration. The concentrations z_1 and z_2 are combined to produce exactly 50% response. A toxic unit (TU) for toxicant i is defined as $\mathrm{TU}_i = z_i/\mathrm{EC50}_i$, where $\mathrm{EC50}_i$ is the (effective) concentration of toxicant i alone, giving 50% response. Simple addition (CA) is characterized by $M = 1$. The condition of $M > 1$ represents antagonism and $M < 1$ indicates synergism. Mixtures that result in 95% confidence interval (CI) for M that overlap 1 are judged to be additive; those with 95% CI that do not overlap 1 are either antagonistic or synergistic in toxicity.

The isoboles for RM and RA can be expressed as follows using the Weibull model for individual toxicants:

Response multiplication, RM

$$\mathrm{TU}_1^{\eta_1} + \mathrm{TU}_2^{\eta_2} = 1, \text{ independent of } Q \tag{2}$$

where

$$\mathrm{TU}_i = \frac{z_i}{\mathrm{EC50}_i} \quad \text{and}$$

η_1, η_2 = slopes of individual Weibull dose-response curves

Response addition, RA

$$\exp(\ln Q \cdot \mathrm{TU}_1^{\eta_1}) + \exp(\ln Q \cdot \mathrm{TU}_2^{\eta_2}) = 1 + Q \tag{3}$$

Note that this isobole depends on the nonresponse level $Q = 1 - P$ where P is the response fraction ($0 \leq P \leq 1$). However, for Q·1 we obtain the above expression for response multiplication.

MATERIALS AND METHODS

Algal incubation

The alga *Raphidocelis subcapitata* (formerly known as *Selenastrum capricornutum*, UTEX 1648) was grown in a 4-L transparent chemostat incubator. The growth medium was supplied continuously by a variable-speed pump. Air agitation was used to achieve adequate mixing. The chemostat reactors were placed in a constant-temperature room at 24 ± 1°C. Light intensity was set at 65 $\mu\mathrm{Em}^{-2}\,\mathrm{s}^{-1}$ (±10%). The growth medium composition is the same as that described by the U.S. Environmental Protection Agency (U.S. EPA) bottle technique [4]. However, according to our previous work [32], $NaNO_3$, K_2HPO_4, and ethylenediaminetetraacetic acid contents were reduced to 12.75 mg/L, 0.52 mg/L, and 30 μg/L, respectively. The dilution rate (D) for the chemostat was set at 0.25/d to ensure a nutrient-limited condition. Quality assurance procedures were conducted routinely by plotting control charts of cell density and pH to verify that steady state was achieved and well maintained.

Toxicity testing

After the algal incubator has reached steady state, toxicity testing was conducted by transferring adequate amounts of algal suspension, dilution water (with growth medium), and toxicants into 300-ml biochemical oxygen demand test bottles. The biochemical oxygen demand bottles were filled up completely with no headspace left [12]. A water seal was provided

Table 2. Physical and chemical properties of the test compounds

Toxicants	Molecular weight	Solubility (μg/L)	Vapor pressure (mmHg)	Boiling point (°C)	Melting point (°C)	Log K_{ow}[a]
Formaldehyde	30.03	Miscible	3,890	−19 (gas)	−92	0.35
Acetaldehyde	44.05	Miscible	902	21	−123	−0.34
Propionaldehyde	58.08	340	317	49	−81	0.59
Butyraldehyde	72.11	71	111	68–77	−97	0.88
Glutaraldehyde	100.12	Miscible	0.6	188	−7	−0.18
2-Hydroxybenzaldehyde	122.12	Miscible	0.593	194–197	−7	1.81
3-Hydroxybenzaldehyde	122.12	28	0.0138	191	99–102	1.29
4-Hydroxybenzaldehyde	122.12	13.8	1.13E-04	—	116	1.35
2-Pyridinecarboxaldehyde	107.11	Soluble	0.568	181	−21	0.44
3-Pyridinecarboxaldehyde	107.11	Soluble	0.568	202	7	0.29
4-Pyridinecarboxaldehyde	107.11	—	0.568	198	−4	0.43
Acetonitrile	41.05	1,000	88.8	81.6	−48	−0.34
Malononitrile	66.06	100	0.2	220	34	−0.6

[a] Log K_{ow} = N-octanol/water partition coefficient (Source: [38] http://www.epa.gov/opptintr/cahp/actlocal/pcgems.html).

to ensure a closed test environment. The bottles then were placed on an orbital shaker operated at 100 rpm. Temperature and light intensity were kept the same as for the algal incubator. The bottle medium used by the U.S. EPA [4] with no ethylenediaminetetraacetic acid content was used for toxicity testing. The dilution water was stripped by nitrogen gas to reduce the dissolved oxygen (DO)level. In addition, the N_2 gas contained 0.5% carbon dioxide as an extra carbon source. The DO level at the beginning of the test was approximately 1 to 2 mg/L. Two response endpoints were used to evaluate the toxicity of toxicants: Dissolved oxygen production (ΔDO) and the algal growth rate calculated based on cell density (number of cells per unit volume). The median effective concentration (EC50) was defined as the toxicant concentration that reduced the final growth rate or the DO production to half of that obtained by the control. The initial inoculated cell density was 15,000 cells/ml and the duration of the test was 48 h. With proper control of inoculum cell density and exposure time, exponential growth was maintained during the entire test period. Therefore, we may conclude that the carbon source was sufficient during the test period.

Toxicity tests for binary mixtures were designed to explore the joint actions between nitriles and aldehydes. These two types of chemicals both are classified as reactive toxicants but with different toxicity mechanisms. Aldehydes are electrophilic nonelectrolytes (Schiff base formation) and nitriles are cyanogenic toxicants [17]. Our previous work indicates that many joint actions between these two kinds of chemicals are synergistic [25–27]. No attempt has been made by previous researchers to explore the combined effects of such organic mixtures on algae.

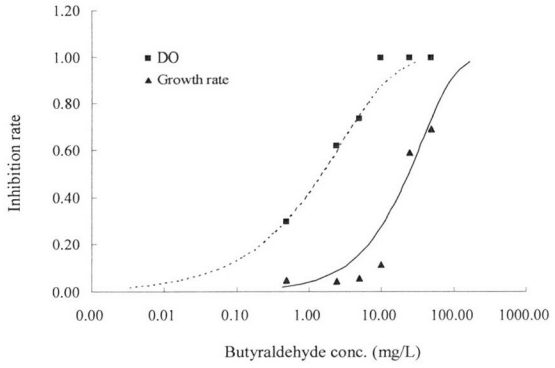

Fig. 1. Concentration–response curves for butyraldehyde. DO = dissolved oxygen.

For algal toxicity tests, the measured concentration is not a practical representation for the amount of toxicant applied in the test because vacuum filtration may cause considerable losses of volatile material. Hence, the toxicant concentrations presented in this work are in the form of nominal concentration. Concentration controls were conducted periodically following exactly the same procedure as described above. The only difference is that no algal inoculum was added to the concentration controls. The prepared controls then were analyzed by the total organic carbon analyzer. The difference between the nominal and measured concentrations was found to be less than 8% with a normal range of 3 to 5%.

Eleven aldehydes and two nitriles were tested in this study. Table 2 lists the names of various organic compounds tested and their physical/chemical properties. Stock solutions of the these compounds were prepared. Before conducting a toxicity test, the concentration of the stock solution was checked using a total organic carbon analyzer. The analytical results were used to define the nominal concentrations of various treatments. All chemicals used were of reagent grade and all tests were performed in triplicate.

RESULTS AND DISCUSSION

Individual toxicity

Figure 1 displays the concentration-response relationships for *R. subcapitata* to butyraldehyde based on two different endpoints (i.e., growth rate and DO production). We find that butyraldehyde exerts a strong inhibitory effect on algal photosynthesis reaction that is related directly to the dissolved oxygen production. The corresponding EC50 value is 1.48 mg/L (0.205×10^{-5} mole/L) and is one order of magnitude smaller than that based on algal growth rate (23.56 mg/L or 3.267×10^{-4} mole/L). Table 3 lists the EC50 values for all thirteen compounds considered in this study. For both DO production and growth rate, 4-hydroxybenzaldehyde is the most toxic compound with EC50 values equal to 1.409 mg/L (0.115×10^{-5} mole/L) and 2.202 mg/L (0.180×10^{-4} mole/L), respectively. Five test compounds, propionaldehyde, butyraldehyde, glutaraldehyde, 3-pyridinecarboxaldehyde, and 3-hydroxybenzaldehyde, displayed stronger toxic effect on DO production than on growth rate. For the other compounds of Table 3, the

Table 3. Median effective concentration values (EC50) based on two different endpoints (growth and dissolved oxygen production)

Response endpoint	Growth rate			DO[a]		
	EC50			EC50		
Chemical	(1×10^{-4}) mol/L	mg/L	ETA[b]	(1×10^{-4}) mol/L	mg/L	ETA[b]
Formaldehyde	1.415	4.249	0.707	0.875	2.627	1.395
Acetaldehyde	1.008	4.439	0.153	0.763	3.359	0.751
Propionaldehyde	4.296	24.95	0.828	1.959	11.38	1.055
Butyraldehyde	3.267	23.56	0.882	0.205	1.480	0.576
Glutaraldehyde	1.318	13.20	0.518	0.395	3.950	0.728
2-Hydroxybenzaldehyde	0.513	6.270	1.093	0.413	5.044	1.659
3-Hydroxybenzaldehyde	6.058	73.98	1.431	1.422	17.37	3.030
4-Hydroxybenzaldehyde	0.180	2.202	0.198	0.115	1.409	1.308
2-Pyridinecarboxaldehyde	2.482	26.59	1.332	2.501	26.79	1.466
3-Pyridinecarboxaldehyde	2.108	22.58	1.093	0.413	4.423	0.619
4-Pyridinecarboxaldehyde	2.504	26.82	1.206	2.183	23.38	1.080
Acetonitrile	193.5	7,943	2.888	144.4	5,926	1.843
Malononitrile	6.23	41.16	0.579	3.108	20.53	0.820

[a] DO = response endpoint based on dissolved oxygen production.
[b] ETA = Weibull slope.

EC50 values based on the DO response also are lower than for growth rate, but the difference is less significant.

Two sets of isomers were tested in the present study to compare the effect of the substituent's position on toxicity. The 3-pyridinecarboxaldehyde is the most toxic compound among the three isomers studied herein, suggesting that *meta* substitution may cause a higher toxicity. This phenomenon is obvious particularly on DO production but is not very significant on algal growth rate. In the case of hydroxybenzaldehydes, the toxicity order in terms of the position of the substituent is *para* > *ortho* > *meta* for both endpoints. This is in accordance with the conclusion drawn by Argese et al. [33] that, for electron withdrawing groups, the substitution at the *para* position seems to be most toxic.

In Table 4, EC50 values are compared with literature data [25,34–37] to evaluate the test sensitivity of the applied technique. For both endpoints (DO and growth rate), our test results reveal apparent superiority over previous data from the conventional algal batch tests [34]. Based on the DO endpoint, our test is 2.5 to 257 times more sensitive than the conventional batch tests. The possible reason for the good test sensitivity is that our tests were conducted in a closed environment and conventional batch tests are open to the atmosphere. In addition, the proposed testing technique (DO endpoint) also is found to be 1 to 100 times more sensitive than the Microtox test. Among all the test species in Table 4, *Daphnia magna* still is the most sensitive test organism to aldehydes. However, the comparison in Table 4 indicates that *R. subcapitata* actually is quite sensitive rather than resistant (an impression based on previous data [34]) to aldehydes. The toxicity orders for selected aldehydes to *R. subcapitata* with respect to different test methods and different endpoints are

Batch test:

Formaldehyde > 2-Hydroxybenzaldehyde > Acetaldehyde > Butyraldehyde

DO endpoint:

Butyraldehyde > Formaldehyde > Acetaldehyde > 2-Hydroxybenzaldehyde

GR endpoint:

Formaldehyde > Acetaldehyde > 2-Hydroxybenzaldehyde > Butyraldehyde

Therefore, the new test technique yields different relative toxicity relationships as compared to that from the conven-

Table 4. Comparisons of algal toxicity test results with literature data[a]

Species chemicals	*Raphidocelis* sp. (this study)		*Raphidocelis* sp.	Microtox	Daphnia
	Growth rate	DO[b]	[34][c]	[25][d]	[35–37][e]
Formaldehyde	4.249	2.627	6.494	6.464	0.029
Acetaldehyde	4.439	3.359	269.74	339.58	0.048
Propionaldehyde	24.95	11.38	—	228.04	—
Butyraldehyde	23.56	1.480	380.18	149.62	0.034
Glutaraldehyde	13.20	3.950	—	3.949	0.075
2-Hydroxybenzaldehyde	6.270	5.044	42.05	—	0.0058

[a] Median effective concentration unit = mg/L; Microtox, Azur Environmental, Carlsbad, CA, USA.
[b] Response endpoint based on dissolved oxygen (DO) production.
[c] Krebs [34].
[d] Chen et al. [25].
[e] Randall et al. [35], Bringmann et al. [36], and Canton et al. [37].

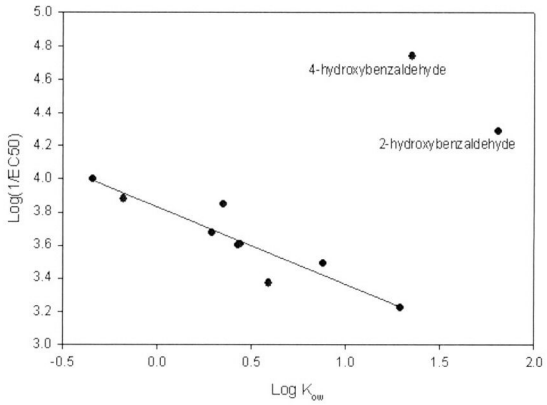

Fig. 2. Correlation between log (1/EC50) and log K_{ow} (K_{ow} = *n*-octanol/water partition coefficient). EC50 = median effective concentration.

tional batch tests. The DO endpoint of the new test method reveals apparent changes in the toxicity order. The main reason is that the DO endpoint reflects the inhibitory effects of toxicants on photosynthesis reactions.

Correlation analyses were conducted to establish the relationship between EC50 values and the *n*-octanol/water partition coefficient (log K_{ow}), considering log(1/EC50) values based on growth rate, a satisfactory linear relationship can be gained versus log K_{ow} by excluding two outliers (2-hydroxybenzaldehyde and 4-hydroxybenzaldehyde) from the analysis. Figure 2 shows that log (1/EC50) values, with EC50 expressed in molar units, correlated well with log K_{ow} with a r^2 value equal to 0.85. The QSAR can be formulated as

$$\log(1/EC50) = 3.83(\pm 0.05) - 0.470(\pm 0.074)\log K_{ow}$$
$$r^2 = 0.85 \quad n = 9 \qquad (4)$$

The satisfactory correlation of toxicity with log K_{ow}, assuming that 2-hydroxybenzaldehyde and 4-hydroxybenzaldehyde are outliers, suggests that hydrophobicity is a major factor in the toxicity.

Combined toxicity

Table 5 displays the additive index (*M*), joint action mode, and the 95% confidence interval for *M*, for various mixtures of aldehydes and nitriles. Among the 10 joint actions related to malononitrile that were associated with a small or mild Weibull slope, four synergistic effects were identified. On the other hand, seven out of 10 joint actions for the steep-slope chemical (acetonitrile) were antagonistic. Furthermore, when different response endpoints were used, the combined effect in terms of additive index and joint action mode might be changed.

The binary toxicity tests in Table 5 were conducted at equitoxic ratio and the mechanisms of toxicity for the two toxicants in a specific mixture, as mentioned previously, were different. Model analyses were conducted to determine the type of joint action between aldehyde and nitrile. The concentration addition (CA) mode obviously is not suitable for describing the joint actions between these two types of toxicants because the CA mode can yield only additive combined effects. Due to the fact that antagonism is the only outcome from NA joint action, NA mode also cannot provide adequate prediction for the observed combined effects.

Predictions based on the RA model are given in Table 5 to compare with the actual experimental observations. Because

Table 5. Additive indices, joint action mode, and 95% confidence intervals for binary toxicity tests

	Growth rate			
	Malononitrile [0.579]		Acetonitrile [2.888]	
Chemicals [slope]	Observation	Prediction	Observation	Prediction
Formaldehyde [0.707]	0.095 [S] 0.160–0.060[a]	0.50 [S]	4.802 [A] 9.475–3.148	1.14 [A]
Acetaldehyde [0.153]	0.953 [+] 1.529–0.392	0.14 [S]	5.430 [A] 7.677–5.426	0.80 [S]
Propionaldehyde [0.828]	0.745 [+] 1.388–0.304	0.56 [S]	6.157 [A] 8.209–4.319	1.18 [A]
Butyraldehyde [0.882]	1.171 [+] 2.221–0.363	0.60 [S]	3.573 [A] 5.597–2.500	1.21 [A]
Glutaraldehyde [0.518]	3.006 [A] 4.782–2.241	0.38 [S]	0.745 [+] 1.392–0.735	1.04 [+]
	Dissolved oxygen			
	Malononitrile [0.820]		Acetonitrile [1.843]	
Chemicals [slope]	Observation	Prediction	Observation	Prediction
Formaldehyde [1.395]	0.278 [S] 0.318–0.243	0.90 [+]	2.015 [A] 2.771–1.525	1.20 [A]
Acetaldehyde [0.751]	0.617 [S] 0.425–0.98	0.64 [S]	1.368 [+] 2.083–0.894	0.96 [+]
Propionaldehyde [1.055]	0.314 [S] 0.516–0.119	0.76 [S]	0.666 [+] 1.070–0.330	1.06 [+]
Butyraldehyde [0.576]	1.102 [+] 1.867–0.727	0.56 [S]	1.928 [A] 3.632–1.056	0.90 [+]
Glutaraldehyde [0.728]	1.867 [A] 2.712–1.359	0.64 [S]	1.930 [A] 2.159–1.726	0.96 [+]

[a] % Confidence intervals + = addition; S = synergism; A = antagonism.

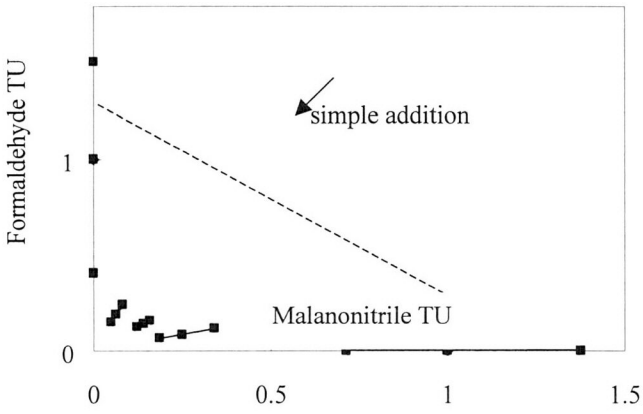

Fig. 3. Malononitrile-formaldehyde isobologram (dissolved oxygen). TU = toxic unit.

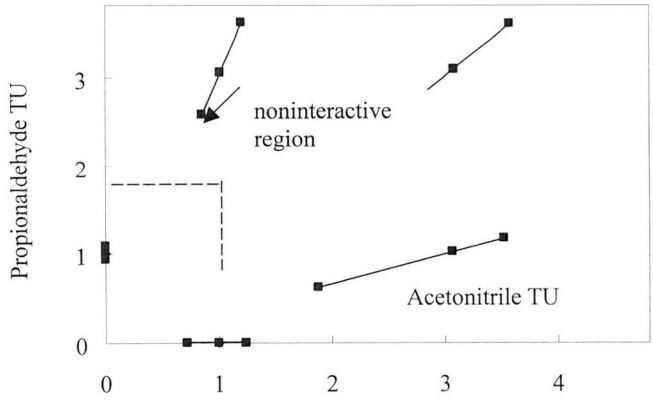

Fig. 5. Acetonitrile-propionaldehyde isobologram (growth rate). TU = toxic unit.

model prediction could not provide the estimation of 95% confidence intervals, an artificial interval of 0.9 to 1.1 was set up for defining the additive effects. From a total of 20 cases of binary tests based on either growth rate or DO production, 10 sets of the combined effect were estimated correctly by the RA mode. Furthermore, three out of the four synergistic actions were predicted successfully (75%). The RA mode thus is considered as more adequate for describing the joint actions between aldehyde and nitrile. In addition, the RA model is capable of forecasting the unexpected hazards from mixtures of dissimilar toxicants that produce synergistic effects. The RM model produces similar predictions as that from the RA model. However, a mixture acting via RA always is more toxic than one acting via RM [29].

By comparing the observed and the predicted additive index values, one finds that the predicted values varied between 0.14 and 1.21 and the actual index values were within the range of 0.095 to 6.157. Such marked differences indicate the possible existence of some interactive joint actions that drastically changed the combined effects as predicted by the noninteractive model. In particular, for the glutaraldehyde-malononitrile mixture, synergistic effects were predicted for both DO and growth rate endpoints but the actual observations were antagonistic.

Interactions for the joint action of malononitrile and formaldehyde are shown in Figure 3 (DO) and Figure 4 (growth rate). Points are shown with one standard deviation. In accordance with Table 5, the observed response for both cases, assuming equitoxic mixtures, reflect synergism with additive indices of 0.278 (0.318, 0.243) and 0.095 (0.160, 0.060) for DO and growth rate, respectively, where numbers in parenthesis are 95% CI. Model predictions for RA and RM are shown in the growth rate plot (Fig. 4). Although the experimental points show more pronounced synergism than the RA model for EC50, it is obvious that the trend is well predicted by this model.

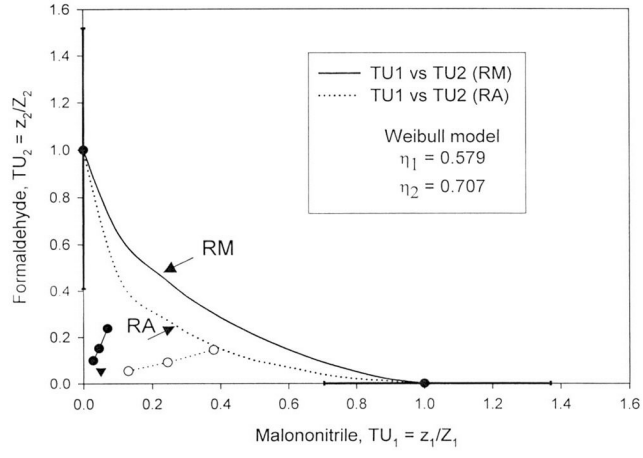

Fig. 4. Malononitrile-formaldehyde isobologram (growth rate). TU = toxic unit, RA = response addition, RM = response multiplication.

Figure 5 displays the isobologram for the combined effects of acetonitrile and propionaldehyde. It is clear that most of the experimental points are located far-off from the noninteractive joint-action region as marked by the dotted line, indicating a strong and antagonistic interactive action. Thus, one of these toxicants acts as an antidote to the other. Considering a typical molar ratio of 490 to 1 for acetonitrile versus propionaldehyde at EC50, this interaction likely is to take place at the organism or cellular level rather than in solution.

Despite all the aforementioned differences between the actual observations and the model predictions, we still may find that the RA model can predict accurately the tendency for toxicants having dissimilar mechanisms to yield synergistic joint actions. For example, as shown in Table 5, malononitrile is more likely to induce greater-than-additive effects because its concentration-response curve displays a rather flat slope. On the other hand, joint actions related to a steep-slope chemical such as the acetonitrile mostly are antagonistic. These experimental observations based on the alga *R. subcapitata* (Table 5) are consistent with our previous conclusions drawn from the *E. coli* and the luminescent bacterial tests [25–27].

CONCLUSION

Toxicity data for 11 aldehydes and two nitriles on *R. subcapitata* are presented. Two kinds of response endpoints were tested in this study: Algal growth rate and dissolved oxygen production. Five test compounds, propionaldehyde, butyraldehyde, glutaraldehyde, 3-pyridinecarboxaldehyde, and 3-hydroxybenzaldehyde, displayed stronger toxic effects on DO production than on growth rate. Therefore, these chemicals have a clear tendency to interfere with photosynthesis reaction. For the rest of the compounds tested, the DO endpoint also shows better sensitivity than growth rate with marginal differences. Compared to literature data, our new test is 2.5 to 257 times more sensitive than the conventional (open) algal batch tests. In addition, our analyses show that different relative toxicity relationships may be observed when different

test methods or different endpoints are applied. The test alga was found to be quite sensitive to aldehydes, and a QSAR relationship was established based on the *n*-octanol/water partition coefficient (log K_{ow}). This study also evaluated the combined effects of aldehydes and nitriles. Four synergistic joint actions related to malononitrile were identified. On the other hand, most of the combined effects between aldehydes and acetonitrile were antagonistic. In general, we find that greater-than-additive effects mainly are associated with toxicants displaying flat dose-response curves and less-than-additive effects may be related to certain steep-slope chemicals. Model analyses show that this mixture toxicity behavior may be due to response addition or response multiplication joint action modes.

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